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DEPARTMENT OF METHODS, REVIEWS, ABSTRACTS, AND BRIEFER ARTICLES

DICHROMATIC ILLUMINATION FOR THE MICROSCOPE

By

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The practise of using monochromatic light in photomicrographic work is widespread. The advantages in the use of such light in conjunction with ocular microscopic examination seem to be less appreciated; while the use of dichromatic illumination, such as that described in this paper, the writer believes to be new.

In using monochromatic light, i.e., light of a given color or wavelength for microscopic examination, the purpose is three-fold: (1) to increase the resolving power of the objective, (2) to secure greater contrast between different parts of the specimen, and (3) to afford relaxation for the eyes. Abbe's formula for ascertaining the resolving power of an objective is to multiply the numerical aperture of the objective by twice the number per inch of the waves of the light employed. Hence it follows that the shorter the wave length of the light, the greater the resolution of the objective. This has an application of appreciable value in connection with the visibility and sharpness of focus of minute objects which seem to lie just upon the border-line of vision. Moreover after working long with white light, green light affords a grateful relaxation to the eyes.

The microscope screen (S, Fig. 1) devised by the writer, has been used with success both for monochromatic and dichromatic illumination. With the former type of illumination the color filter is inserted in the slide (C1, Figs. 1 and 2) and the light obtained from the arc-lamp (A1, Figs. 1 and 2).

For the examination of minute structures in the protozoan cell, and of the pigment granules in the cortex of mammalian hairs, green or blue illumination was found to be excellent. The former is more restful to the eyes, especially when making protracted examinations. It is, moreover, of greater luminosity, and hence permits of greater ease in focussing the specimen. In the examination of protozoa the writer's practise is to focus the object by the green light, and then exchange this for the blue. Violet light was found unsatisfactory, because of its lack of luminosity. Certain

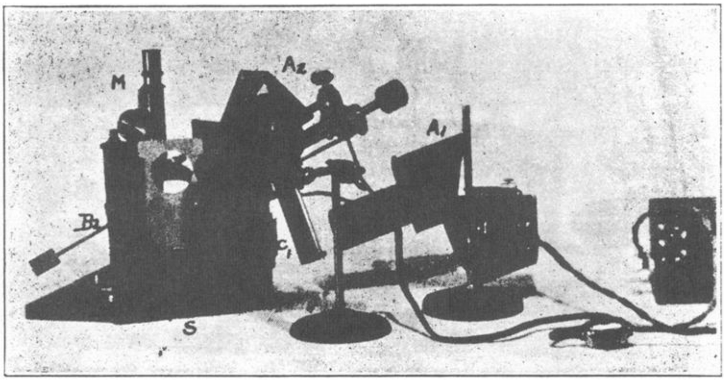

FIG. 1

Assemblage of apparatus for dichromatic illumination. Microscope (M) in position behind the screen (S), which bears slides (C_1 and C_2) for supporting the color filters. Illumination of the object above and below is secured from the two arc lamps (A_1 and A_2).

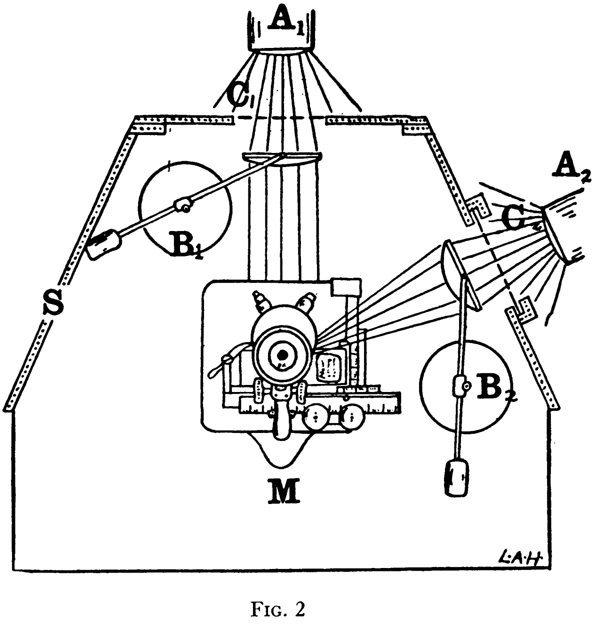

FIG. 2

Diagrammatic view of apparatus for dichromatic illumination, from above. A_1 and A_2 arc lamps, for transmitted and reflected light respectively; B_1 and B_2, plano-convex condensers; the former to deliver practically parallel rays to mirror, the latter to illumine object from above; C_1 and C_2, apertures for color filters; M, microscope. The screen (S) is stippled.

stained structures show up well with monochromatic light, particularly if the color used be complementary to the one used as stain.

By employing a new type of illumination, i.e., dichromatic, it is possible by means of the illumination alone to invest certain portions of the specimen with one color, and other portions with another, for the purpose of bringing out thus by contrast the forms or relationships of the two. The principle of dichromatic illumination is to illuminate the microscope field and the transparent portions of the specimen with one color, by means of transmitted light, and the more dense, or opaque portions of the specimen with another color, by means of reflected light, and to secure the maximum contrast between the structures so illuminated by using complementary colors.

Such a result has been secured by means of the apparatus shown in Figs. 1 and 2 (whose letterings are similar). The microscope (M) is sufficiently protected from all rays of light save those desired, by the screen (S), which bear two slides (C1 and C2). In these slides are apertures wherein can be placed color filters. Filter C1 delivers to the microscope mirror light of one color for illuminating the field and the transparent portions of the specimen, and filter C2 delivers light of another color which is focussed upon the specimen from above. The transparent portions of the specimen are therefore viewed by transmitted light of one color, the opaque portions by reflected light of another color, and the translucent portions by a combination of these two. Slide C1 bearing its filter can be moved laterally, and slide C2 vertically. The positions which the filters can be made to occupy, together with the various positions of the microscope within the screen, make it possible to secure light from any of the angles useful in microscopic work. Illumination is furnished by two movable arc-lamps, A1 for delivering light to the mirror, and A2 for lighting the specimen from above.

Within the microscope screen two movable plano-convex condensers are used; one of long focus (B1), for delivering to the mirror virtually parallel rays; the other (B2) of short focus for condensing the light upon the specimen from above.

With such an apparatus the writer has secured good results in the examination of mammalian hairs, for the detection of delicate scalation. With further experimentation the use of such lighting may possibly be widened.

Color filters for use in photomicrographic work can be purchased and used in the screen. Good filters can be made, however, in the laboratory by developing out unexposed lantern slides, or dry plates, and then staining the clear gelatin film with the desired colors. Care must be taken to secure color filters that will allow only one color of light to pass through them, i.e., they must furnish, as nearly as possible, monochromatic light. Some of the stains, in aqueous solution, which have been recommended for this purpose are: (1) Yellow—a saturated solution of picric acid. This absorbs almost completely the blue and violet end of the spectrum. (2) Green—methyl green. The absorption spectra varies with the depth of the color. (3) Copper sulphate. This absorbs the red almost entirely. Methyl blue is fairly good. (4) Red—Safranin.

The procedure in making color filters from lantern slide plates is to develop and fix the unexposed plates and then to allow the gelatin film to soften by placing the plates in a bath of slightly warm water, say 80 degrees F. for a few minutes. They may then be removed to baths of the different stains, and allowed to soak for an hour or so, or until the gelatin is evenly stained. It was found best to make up a series of saturated aqueous solutions of the stains, and from these gradually to increase the depth of color of the various baths until the desired depth was secured in the gelatin film. The optimum for color filters is the greatest depth of color which one can use and still secure sufficient luminosity for good focus. The depth of the color of the filter giving the best results will depend upon the brightness of the illuminant. This latter should give a strong, white light. Such a light, with a spectrum very much like that of sunlight, can be obtained from the electric arc.